

[0220] Peptide aptamers from combinatorial libraries can be dominant inhibitors of gene function. For example, peptide aptamers can be used to inhibit the pathway of interest in the cells expressing the peptide aptamers and to identify mutants with phenotypes of interest. C R Geyer, A Colman-Lerner, R Brent; PNAS 96 (1999)8567-8572.

[0221] A biologically active compound can be conjugated to an affinity reagent to produce a chimeric protein that both binds the target and produces a desirable biological functionality. For example, a binding reagent could be fused to the functional domain of the Bt protein (domain 2) to produce a novel protein capable of binding proteins other than its target protein (e.g., aminopeptidase), producing septicemia similar to that seen with Bt toxicity. In addition, the aptamer itself may have biological activity in addition to the affinity to the expressed cDNA protein.

[0222] Promoter sequence(s) and other genetic elements including but not limited to transcriptional regulatory elements associated with one or more of the disclosed nucleotide sequences can also be obtained using the disclosed nucleotide sequences provided herein. In one embodiment, such sequences are obtained by incubating EST nucleic acid molecules or preferably fragments thereof with members of genomic libraries and recovering clones that hybridize to the EST nucleic acid molecule or fragment thereof. In a second embodiment, methods of "chromosome walking," or inverse PCR may be used to obtain such sequences (Frohman, et al., *Proc. Natl. Acad. Sci. (U.S.A.)* 85:8998-9002 (1988); Ohara, et al., *Proc. Natl. Acad. Sci. (U.S.A.)* 86: 5673-5677 (1989); Pang et al., *Biotechniques*, 22(6); 1046-1048 (1977); Huang et al., *Methods Mol. Biol.* 69: 89-96 (1977); Hard et al., *Methods Mol. Biol.* 58: 293-301 (1996)). In one embodiment, the disclosed ESTs are used to identify cDNAs whose analogous genes contain promoters with desirable expression patterns. Isolation and functional analysis of the 5' flanking promoter sequences of these genes from genomic libraries, for example, using genomic screening methods and PCR techniques would result in the isolation of useful promoters and transcriptional regulatory elements. These methods are known to those of skill in the art and have been described (See for example Birren et al., *Genome Analysis: Analyzing DNA*, 1, (1997), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). Promoters obtained utilizing the ESTs of the present invention could also be modified to affect their control characteristics. Examples of such modifications would include but are not limited to enhancer sequences as reported by Kay et al., *Science* 236:1299 (1987).

[0223] In one aspect of the present invention, an evaluation can be conducted to determine whether a particular mRNA molecule is present. One or more of the nucleic acid molecules of the present invention, preferably one or more of the EST nucleic acid molecules of the present invention are utilized to detect the presence or quantity of the mRNA species. Such molecules are then incubated with cell or tissue extracts of a plant under conditions sufficient to permit nucleic acid hybridization. The detection of double-stranded probe-mRNA hybrid molecules is indicative of the presence of the mRNA; the amount of such hybrid formed is proportional to the amount of mRNA. Thus, such probes may be used to ascertain the level and extent of the mRNA production in a plant's cells or tissues. Such nucleic acid hybridization may be conducted under quantitative conditions (thereby providing a numerical value of the amount of the mRNA present). Alternatively, the assay may be conducted as a qualitative

assay that indicates either that the mRNA is present, or that its level exceeds a user set, predefined value.

[0224] A principle of in situ hybridization is that a labeled, single-stranded nucleic acid probe will hybridize to a complementary strand of cellular DNA or RNA and, under the appropriate conditions, these molecules will form a stable hybrid. When nucleic acid hybridization is combined with histological techniques, specific DNA or RNA sequences can be identified within a single cell. An advantage of in situ hybridization over more conventional techniques for the detection of nucleic acids is that it allows an investigator to determine the precise spatial population (Angerer et al., *Dev. Biol.* 101: 477-484 (1984); Angerer et al., *Dev. Biol.* 112: 157-166 (1985); Dixon et al., *EMBO J.* 10: 1317-1324 (1991)). In situ hybridization may be used to measure the steady-state level of RNA accumulation. It is a sensitive technique and RNA sequences present in as few as 5-10 copies per cell can be detected (Hardin et al., *J. Mol. Biol.* 202: 417-431. (1989)). A number of protocols have been devised for in situ hybridization, each with tissue preparation, hybridization, and washing conditions (Meyerowitz, *Plant Mol. Biol. Rep.* 5: 242-250 (1987); Cox and Goldberg, In: *Plant Molecular Biology: A Practical Approach* (ed. C. H. Shaw), pp. 1-35. IRL Press, Oxford (1988); Raikhel et al., *In situ RNA hybridization in plant tissues*. In *Plant Molecular Biology Manual*, vol. B9: 1-32. Kluwer Academic Publisher, Dordrecht, Belgium (1989)).

[0225] In situ hybridization also allows for the localization of proteins within a tissue or cell (Wilkinson, *In Situ Hybridization*, Oxford University Press, Oxford (1992); Langdale, *In Situ Hybridization* 165-179 In: *The Maize Handbook*, eds. Freeling and Walbot, Springer-Verlag, New York (1994)). It is understood that one or more of the molecules of the present invention, preferably one or more of the EST nucleic acid molecules of the present invention or one or more of the antibodies of the present invention may be utilized to detect the expression level or pattern of a protein or mRNA thereof by in situ hybridization.

[0226] Fluorescent in situ hybridization also enables the localization of a particular DNA sequence along a chromosome which is useful, among other uses, for gene mapping, following chromosomes in hybrid lines or detecting chromosomes with translocations, transversions or deletions. In situ hybridization has been used to identify chromosomes in several plant species (Griffon et al., *Plant Mol. Biol.* 17: 101-109 (1991); Gustafson et al., *Proc. Nat'l. Acad. Sci. (U.S.A.)* 87: 1899-1902 (1990); Mukai and Gill, *Genome* 34: 448-452. (1991); Schwarzacher and Heslop-Harrison, *Genome* 34: 317-323 (1991); Wang et al., *Jpn. J. Genet.* 66: 313-316 (1991); Parra and Windle, *Nature Genetics*, 5: 17-21 (1993)). It is understood that the nucleic acid molecules of the present invention may be used as probes or markers to localize sequences along a chromosome.

[0227] It is also understood that one or more of the molecules of the present invention, preferably one or more of the EST nucleic acid molecules of the present invention or one or more of the antibodies of the present invention may be utilized to detect the expression level or pattern of a protein or mRNA thereof by in situ hybridization.

[0228] Further, it is also understood that any of the nucleic acid molecules of the present invention may be used as marker nucleic acids and or probes in connection with methods that require probes or marker nucleic acids. As used herein, a probe is an agent that is utilized to determine an